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Bacteria detection with high-frequency gravimetric biosensors based on AlN thin film resonators

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Abstract

Gravimetric sensors based on shear-mode resonators are suitable for in-liquid detection of biological species because their quality factors barely decrease during in-liquid operation. However, we have found that in the particular case of large ligands, such as bacteria, the transmission of the surface movement to them appears to be more efficient when movement takes place normal to the surface (longitudinal modes) instead of to parallel to it (shear modes). In this work, we succeeded in detecting bacteria with AlN-based bulk acoustic wave solidly mounted resonators operating in longitudinal modes at 2 GHz that we were unable to detect with shear modes.

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*Keywords:*Gravimetric sensing, high frequeny resonators, bacteria detection

1. Introduction

Rapid identification of whole cell bacteria can be critical for clinical diagnosis and treatment, food safety, public health, or security. To avoid sample treatment, manipulation and complex detection techniques, a bacterial detection test easily accessible by non-expert users would be desirable. Label free biosensors, and particularly acoustic biosensors, are a good choice to address this challenge. The increasing number of scientific reports dealing with the use of this kind of sensors for whole cell detection highlights the growing interest for this technology [1].

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In previous works, we assessed the performance of AlN-based solidly mounted resonators (SMRs) operating in the shear mode at 1.4 GHz as gravimetric sensors for the in-liquid detection of different biomolecules (up to 150 kDa). These resonators displayed a mass sensitivity of 1800 kHz·cm^2/pg. A streptavidin-biotin functionalization protocol of the resonator active area allowed us to detect IgG and thrombin. In the latter case, we succeeded in detecting thrombin concentrations as low as 4 nM. However, we were unable to detect whole cell bacteria. Conversely, some groups reported the detection of this kind of target with conventional quartz crystal microbalance (QCM) operating between 5 MHz and 10 MHz (with its harmonics), but with a limit of detection too high. QCMs operate in shear mode like SMRs and detection is achieved by tracking the changes experienced by the resonant frequency and the dissipation mechanisms (quality factor Q). Changes in dissipation are related to viscoelastic properties but how the detection works is still unclear [2]. Other groups detected mammalian cells with QCMs and Love-surface acoustic wave sensors operating at 500 MHz, attributing the detection process to the variation of the viscoelastic and mechanical properties of the QCM surface [3]. Dultsev et al [4] detected the release of virus specifically bound with antibodies to the QCM surface when the applied power is increased. They build a model explaining that rigid particles, as virus or bacteria, cannot be detected under shear vibration because the molecules involved in particle attachment move parallel to the surface and are unable to drag cells. In this paper we propose to detect bacteria with SMRs operating in longitudinal mode at 2.2 GHz. Whole bacteria are detected by monitoring the shift of resonant frequency without any dissipation change.

2. Experimental

The core of the sensing technology is a piezoelectric resonator made of an AlN thin film sandwiched between two Ir electrodes and deposited on insulating acoustic mirrors. Two types of resonators have been used: shear-mode SMRs based on AlN films with tilted grains and longitudinal-mode ones made with c-axis oriented AlN films, with resonant frequencies of 1.4 GHz and 2.5 GHz, respectively. Details of the fabrication procedure can be found in [5].

After fabrication, the devices were diced into chips and their active surface (top electrode covered with sputtered SiO_2) was functionalized using a standard APTES ((3-Aminopropyl) triethoxysilane)-GA (glutaraldehyde) functionalization protocol [6] slightly modified. The aim of the functionalization process was to cover the entire active area of the resonator with receptors (antibodies) tightly bound to its surface with the right orientation, so that they exposed their active side to the target. To achieve this goal we took advantage of the high affinity of streptavidin to biotin, and the fact that any receptor can be biotin-modified at any position within the molecule. The reagents used are APTES 2% (Sigma-Aldrich) in ethanol (Merck), glutaraldehyde (Sigma-Aldrich), streptavidin 10 µg/ml (Sigma-Aldrich), and antibodies against legionella and salmonella (Sigma-Aldrich).

Sensors were initially characterized by measuring their electrical impedance from 10 MHz to 10 GHz with an Agilent N5230A network analyzer using calibrated RF probes for on-wafer contacting. For the accurate tracking of the resonant frequency during bio-detection experiments, the maximum of the real part of the admittance was fitted to an eighth degree polynomial in a narrow frequency interval. The roots of its first derivative were calculated and the resonant frequency identified. This procedure was implemented in a LabView® application which allows a measurement of the frequency with 1 kHz accuracy each 3 seconds.

3. Results

In a first attempt to detect bacteria with an SMR, we used a shear-mode resonator functionalized with the specific antibodies. When the solution containing a high concentration of legionella bacteria was fed to the resonator, no appreciable frequency shift took place (see figure 1(a)). Bacteria binding was verified by ELISA analysis and, therefore, we concluded that the bacteria did not effectively charge the resonator, probably because their size is too large to be moved by the oscillating surface. To verify this hypothesis bacteria were solubilized breaking them into small pieces. Then, we repeated the detection experiment and observed a slight variation in the resonant frequency (figure 1(b)).

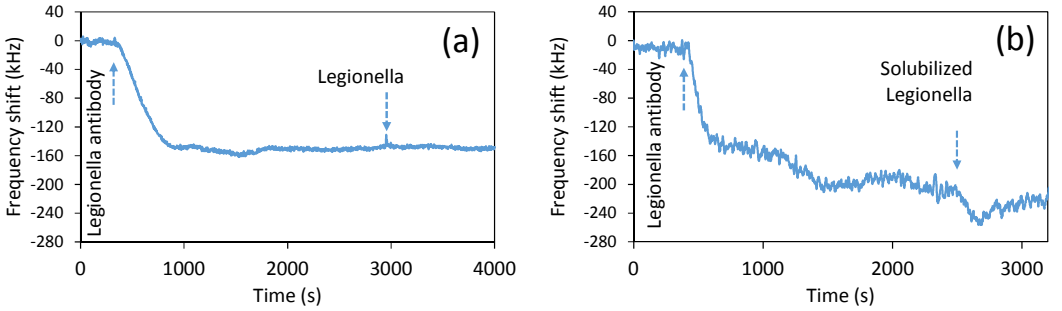

Fig. 1. Time evolution of the resonant frequency of a shear mode resonator when exposed to legionella antibody and legionella for (a) whole bacteria and (b) solubilized bacteria.

In a second attempt to gravimetric detection of bacteria we use a high-quality SMR made with perfectly c-axis oriented AlN film working in longitudinal mode at 2.2 GHz of resonant frequency. After functionalizing its surface with antibodies, a highly concentrated solution of legionella bacteria was fed into the fluidic system, producing a strong variation of the resonant frequency as shown in figure 2(a) (red-line). To verify that this response was not due to unspecific binding or to some change in the liquid properties, we repeated the experiment using salmonella antibody, obtaining a perfect blank (see figure 2(a), blue line). This indicated that the frequency shift was truly due to the binding of legionella bacteria to the surface of the resonator. In addition to the resonant frequency, the quality factor at resonant frequency was monitored. Figure 2(b) shows that quality factor, which is mainly related to changes in the viscosity of the liquid, barely varies in both positive and negative tests. This confirms that the frequency shift is mainly due to a variation of mass and not to changes in viscosity or other loss mechanism that could affect the value of the resonant frequency.

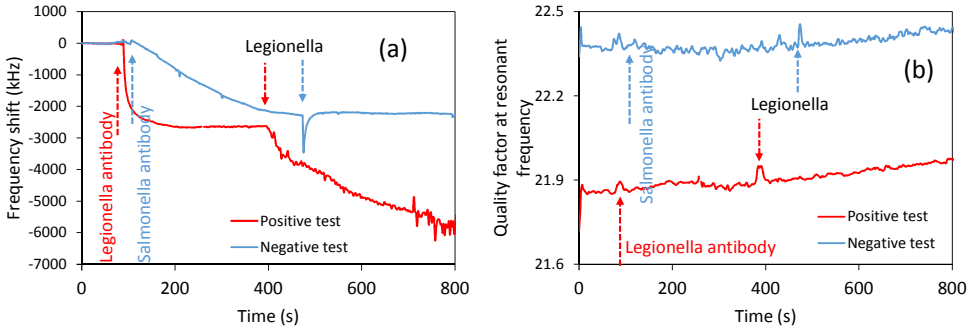

Fig. 2. Time evolution of the frequency shift (a) and the quality factor (b) in a positive (red) and negative (blue) detection of legionella bacteria using a longitudinal mode resonator.

4. Discussion

When a resonator operates in a fluid (gas or liquid), part of its acoustic energy is radiated to the medium. In shear mode resonators, the transmission of energy depends on the viscosity of the fluid, since the movement of the top of the resonator is parallel to the fluid interface and energy can be only radiated if the different layers of the fluid have certain adhesion (high viscosity). For gases or liquids of low viscosity, this adhesion between fluid layers is very small, being the acoustic radiation very scarce. Conversely, in longitudinal mode resonators the top of the resonators moves perpendicular to the fluid interface, displacing the fluid and generating a pressure wave (sound wave). In this case, the radiated energy depends on the density and elastic constants of the fluid (compressibility). For these reasons, shear-mode resonators suffer a lower reduction of the quality factor when operating in liquids. A typical SMR with

tilted AlN operating in a PBS buffer suffers a reduction in the quality factor of its shear mode resonance of around 15%, dropping from 200 (in air) to 170 (in liquid), whereas the quality factor of the same SMR operating in the longitudinal mode experiences a reduction of 96%, dropping from 700 (in air) to 22 (in liquid). However, in spite of the low Q value, it is sufficient to enable the tracking of the resonant frequency with the method described in the experimental section.

The obtained results suggest the binding of bacteria to surface of the resonator is not effective. For gravimetric detection, the bacteria must load effectively the resonator; in other words, they must move coherently with the surface in order to increase the effective mass of the resonator, the compliance of the binding chains playing a fundamental role. The bond of the bacteria to the surface involves the binding with a silane group followed by a glutaraldehyde molecule, a streptavidin protein and a biotinylated antibody. Our hypothesis is that this chain of molecules has a large compliance in the direction parallel to the surface, being unable to transmit the surface movement to the bacteria at such high working frequencies (1.4 GHz). In addition, large size bacteria suffer from the inertia and the friction force with the liquid that impedes their movement. On the contrary, under the transverse movement imposed by longitudinal modes, the bonding chain seems to be rigid enough to transmit the movement of the surface to the bacteria, meaning that the bond is less compliant and the bacteria effectively “charges” the resonator. In this case, both inertia and viscous forces influences the bacteria movement. Figure 3 shows a sketch of these situations.

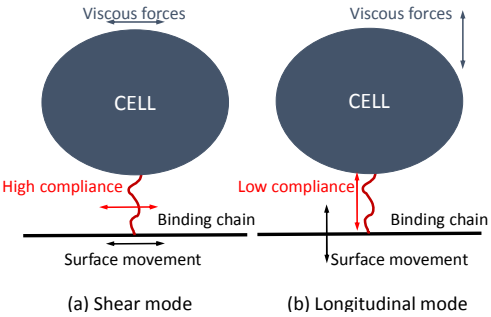

Figure 3: Simple model of the interaction bacteria-surface for (a) shear mode and (b) longitudinal mode resonator operation.

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